(54) Title: FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION

(57) Abstract

An altered antibody chain is produced in which the CDR's of the variable domain of the chain are derived from a first mammalian species. The framework-encoding regions of DNA encoding the variable domain of the first species are mutated so that the mutated framework-encoding regions encode a framework derived from a second different mammalian species. The or each constant domain of the antibody chain, if present, are also derived from the second mammalian species. An antibody which is capable of binding to human CD4 antigen is also provided together with a pharmaceutical composition comprising the antibody.

FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION

   The present invention relates to altered antibodies and
their preparation. The invention is typically applicable
to the production of humanised antibodies.

5   Antibodies typically comprise two heavy chains linked
together by disulphide bonds and two light chains. Each
light chain is linked to a respective heavy chain by
disulphide bonds. Each heavy chain has at one end a
variable domain followed by a number of constant domains.

10 Each light chain has a variable domain at one end and a
constant domain at its other end. The light chain variable
domain is aligned with the variable domain of the heavy
chain. The light chain constant domain is aligned with the
first constant domain of the heavy chain. The constant

15 domains in the light and heavy chains are not involved
directly in binding the antibody to antigen.

   The variable domains of each pair of light and heavy
chains form the antigen binding site. The domains on the
light and heavy chains have the same general structure and

20 each domain comprises a framework of four regions, whose
sequences are relatively conserved, connected by three
complementarity determining regions (CDRs). The four
framework regions largely adopt a beta-sheet conformation
and the CDRs form loops connecting, and in some cases

25 forming part of, the beta-sheet structure. The CDRs are
held in close proximity by the framework regions and, with
the CDRs from the other domain, contribute to the formation
of the antigen binding site.

   The preparation of an altered antibody in which the CDRs

30 are derived from a different species than the framework of
the antibody's variable domains is disclosed in
EP-A-0239400. The CDRs may be derived from a rat or mouse
monoclonal antibody. The framework of the variable
domains, and the constant domains, of the altered antibody

35 may be derived from a human antibody. Such a humanised

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antibody elicits a negligible immune response when
administered to a human compared to the immune response
mounted by a human against a rat or mouse antibody.
Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404.

5   We have now devised a new way of preparing an altered
antibody. In contrast to previous proposals, this involves
altering the framework of a variable domain rather than the
CDRs. This approach has the advantages that it does not
require a pre-existing cDNA encoding, for example, a human
10 framework to which to reshape and that it is technically
easier than prior methodologies.

     Accordingly, the present invention provides a process
for the preparation of an antibody chain in which the CDRs
of the variable domain of the antibody chain are derived
15 from a first mammalian species and the framework of the
variable domain and, if present, the or each constant
domain of the antibody chain are derived from a second
different mammalian species, which process comprises:

     (i) mutating the framework-encoding regions of DNA
20 encoding a variable domain of an antibody chain of the said
first species such that the mutated framework-encoding
regions encode the said framework derived from the said
second species; and

     (ii) expressing the said antibody chain utilising the
25 mutated DNA from step (i).

     A variable domain of either or both chains of an
antibody can therefore be altered by:

     (a) determining the nucleotide and predicted amino acid
sequence of a variable domain of a selected antibody chain
30 of the said first species;

     (b) determining the antibody framework to which the
framework of the said variable domain is to be altered;

     (c) mutating the framework-encoding regions of DNA
encoding the said variable domain such that the mutated

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framework-encoding regions encode the framework determined
upon in step (b);

(d) linking the mutated DNA obtained in step (c) to DNA
encoding a constant domain of the said second species and
5   cloning the DNA into an expression vector; and

(e) introducing the expression vector into a compatible
host cell and culturing the host cell under such conditions
that antibody chain is expressed.

The antibody chain may be co-expressed with a
10   complementary antibody chain. At least the framework of
the variable domain and the or each constant domain of the
complementary chain generally are derived from the said
second species also. A light chain and a heavy chain may
be co-expressed. Either or both chains may have been
15   prepared by the process of the invention. Preferably the
CDRs of both chains are derived from the same selected
antibody. An antibody comprising both expressed chains can
be recovered.

The antibody preferably has the structure of a natural
20   antibody or a fragment thereof. The antibody may therefore
comprise a complete antibody, a (Fab')$_2$ fragment, a Fab
fragment, a light chain dimer or a heavy chain. The
antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4
IgM, IgA, IgE or IgD. Alternatively, the antibody may be a
25   chimaeric antibody of the type described in WO 86/01533.

A chimaeric antibody according to WO 86/01533 comprises
an antigen binding region and a non-immunoglobulin region.
The antigen binding region is an antibody light chain
variable domain or heavy chain variable domain. Typically,
30   the chimaeric antibody comprises both light and heavy chain
variable domains. The non-immunoglobulin region is fused
at its C-terminus to the antigen binding region. The non-
immunoglobulin region is typically a non-immunoglobulin
protein and may be an enzyme region, a region derived from
35   a protein having known binding specificity, from a protein

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toxin or indeed from any protein expressed by a gene. The
two regions of the chimaeric antibody may be connected via
a cleavable linker sequence.

   The invention is preferably employed to humanise an
5 antibody, typically a monoclonal antibody and, for example,
a rat or mouse antibody. The framework and constant
domains of the resulting antibody are therefore human
framework and constant domains whilst the CDRs of the light
and/or heavy chain of the antibody are rat or mouse CDRs.
10 Preferably all CDRs are rat or mouse CDRs. The antibody
may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM;
IgA; IgE or IgD carrying rat or mouse CDRs.

   The process of the invention is carried out in such a
way that the resulting antibody retains the antigen binding
15 capability of the antibody from which it is derived. An
antibody is reshaped according to the invention by mutating
the framework-encoding regions of DNA coding for the
variable domains of the antibody. This antibody and the
reshaped antibody should both be capable of binding to the
20 same antigen.

   The starting antibody is typically an antibody of a
selected specificity. In order to ensure that this
specificity is retained, the variable domain framework of
the antibody is preferably reshaped to about the closest
25 variable domain framework of an antibody of another
species. By "about the closest" is meant about the most
homologous in terms of amino acid sequences. Preferably
there is a homology of at least 50% between the two
variable domains.

30   There are four general steps to reshape a monoclonal
antibody. These are:

      (1) determining the nucleotide and predicted amino acid
sequence of the starting antibody light and heavy chain
variable domains;

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(2) designing the reshaped antibody, i.e. deciding
which antibody framework region to use during the reshaping
process;

(3) the actual reshaping methodologies/techniques; and

5   (4) the transfection and expression of the reshaped
antibody.

These four steps are explained below in the context of
humanising an antibody. However, they may equally well be
applied when reshaping to an antibody of a non-human

10 species.

Step 1: Determining the nucleotide and predicted amino acid
        sequence of the antibody light and heavy chain
        variable domains

To reshape an antibody only the amino acid sequence of

15 antibody's heavy and light chain variable domains needs to
be known. The sequence of the constant domains is
irrelevant because these do not contribute to the reshaping
strategy. The simplest method of determining an antibody's
variable domain amino acid sequence is from cloned cDNA

20 encoding the heavy and light chain variable domain.

There are two general methods for cloning a given
antibody's heavy and light chain variable domain cDNAs: (1)
via a conventional cDNA library, or (2) via the polymerase
chain reaction (PCR). Both of these methods are widely

25 known. Given the nucleotide sequence of the cDNAs, it is a
simple matter to translate this information into the
predicted amino acid sequence of the antibody variable
domains.

30 Step 2: Designing the reshaped antibody

There are several factors to consider in deciding which
human antibody sequence to use during the reshaping. The
reshaping of light and heavy chains are considered
independently of one another, but the reasoning is

35 basically similar for each.

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This selection process is based on the following
rationale: A given antibody's antigen specificity and
affinity is primarily determined by the amino acid sequence
of the variable region CDRs. Variable domain framework
5   residues have little or no direct contribution. The
primary function of the framework regions is to hold the
CDRs in their proper spacial orientation to recognize
antigen. Thus the substitution of rodent CDRs into a human
variable domain framework is most likely to result in
10 retention of their correct spacial orientation if the human
variable domain is highly homologous to the rodent variable
domain from which they originated. A human variable domain
should preferably be chosen therefore that is highly
homologous to the rodent variable domain(s).

15 A suitable human antibody variable domain sequence can
be selected as follows:
1. Using a computer program, search all available protein
   (and DNA) databases for those human antibody variable
   domain sequences that are most homologous to the rodent
20   antibody variable domains. This can be easily
   accomplished with a program called FASTA but other
   suitable programs are available. The output of a
   suitable program is a list of sequences most homologous
   to the rodent antibody, the percent homology to each
25   sequence, and an alignment of each sequence to the
   rodent sequence. This is done independently for both
   the heavy and light chain variable domain sequences.
   The above analyses are more easily accomplished if
   customized sub-databases are first created that only
30   include human immunoglobulin sequences. This has two
   benefits. First, the actual computational time is
   greatly reduced because analyses are restricted to only
   those sequences of interest rather than all the
   sequences in the databases. The second benefit is that,
35   by restricting analyses to only human immunoglobulin

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sequences, the output will not be cluttered by the
presence of rodent immunoglobulin sequences. There are
far more rodent immunoglobulin sequences in databases
than there are human.

5 2. List the human antibody variable domain sequences that
have the most overall homology to the rodent antibody
variable domain (from above). Do not make a distinction
between homology within the framework regions and CDRs.
Consider the overall homology.

10 3. Eliminate from consideration those human sequences that
have CDRs that are a different length than those of the
rodent CDRs. This rule does not apply to CDR 3, because
the length of this CDR is normally quite variable.
Also, there are sometimes no or very few human sequences

15   that have the same CDR lengths as that of the rodent
antibody. If this is the case, this rule can be
loosened, and human sequences with one or more
differences in CDR length can be allowed.

4. From the remaining human variable domains, the one is
20   selected that is most homologous to that of the rodent.

5. The actual reshaped antibody (the end result) should
contain CDRs derived from the rodent antibody and a
variable domain framework from the human antibody chosen
above.

25 Step 3: The actual reshaping methodologies/techniques
A cDNA encoding the desired reshaped antibody is
preferably made beginning with the rodent cDNA from which
the rodent antibody variable domain sequence(s) was
originally determined. The rodent variable domain amino

30 acid sequence is compared to that of the chosen human
antibody variable domain sequence. The residues in the
rodent variable domain framework are marked that need to be
changed to the corresponding residue in the human to make
the rodent framework identical to that of the human

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framework. There may also be residues that need adding to
or deleting from the rodent framework sequence to make it
identical to that of the human.

   Oligonucleotides are synthesised that can be used to
5 mutagenize the rodent variable domain framework to contain
the desired residues. Those oligonucleotides can be of any
convenient size. One is normally only limited in length by
the capabilities of the particular synthesizer one has
available. The method of oligonucleotide-directed _in vitro_
10 mutagenesis is well known.

   The advantages of this method of reshaping as opposed to
splicing CDRs into a human framework are that (1) this
method does not require a pre-existing cDNA encoding the
human framework to which to reshape and (2) splicing CDRs
15 is technically more difficult because there is usually a
large region of poor homology between the mutagenic oligo-
nucleotide and the human antibody variable domain. This is
not so much a problem with the method of splicing human
framework residues onto a rodent variable domain because
20 there is no need for a pre-existing cDNA encoding the human
variable domain. The method starts instead with the rodent
cDNA sequence. Also, splicing framework regions is
technically easier because there is a high degree of
homology between the mutagenic oligonucleotide and the
25 rodent variable domain framework. This is true because a
human antibody variable domain framework has been selected
that is most homologous to that of the rodent.

   The advantage of the present method of reshaping as
opposed to synthesizing the entire reshaped version from
30 scratch is that it is technically easier. Synthesizing a
reshaped variable domain from scratch requires several more
oligonucleotides, several days more work, and technical
difficulties are more likely to arise.

<u>Step 4</u>: <u>The transfection and expression of the reshaped</u>
<u>antibody</u>

Following the mutagenesis reactions to reshape the
antibody, the cDNAs are linked to the appropriate DNA
5 encoding light or heavy chain constant region, cloned into
an expression vector, and transfected into mammalian cells.
These steps can be carried out in routine fashion. A
reshaped antibody may therefore be prepared by a process
comprising:
10   a)   preparing a first replicable expression vector
including a suitable promoter operably linked to a DNA
sequence which encodes at least a variable domain of an Ig
heavy or light chain, the variable domain comprising
framework regions from a first antibody and CDRs comprising
15 at least parts of the CDRs from a second antibody of
different specificity;
        b)   if necessary, preparing a second replicable
expression vector including a suitable promoter operably
linked to a DNA sequence which encodes at least the
20 variable domain of a complementary Ig light or heavy chain
respectively;
        c)   transforming a cell line with the first or both
prepared vectors; and
        d)   culturing said transformed cell line to produce
25 said altered antibody.

Preferably the DNA sequence in step a) encodes both the
variable domain and the or each constant domain of the
antibody chain, the or each constant domain being derived
from the first antibody. The antibody can be recovered and
30 purified. The cell line which is transformed to produce
the altered antibody may be a Chinese Hamster Ovary (CHO)
cell line or an immortalised mammalian cell line, which is
advantageously of lymphoid origin, such as a myeloma,
hybridoma, trioma or quadroma cell line. The cell line may
35 also comprise a normal lymphoid cell, such as a B-cell,

which has been immortalised by transformation with a virus,
such as the Epstein-Barr virus. Most preferably, the
immortalised cell line is a myeloma cell line or a
derivative thereof.

5   Although the cell line used to produce the altered
antibody is preferably a mammalian cell line, any other
suitable cell line, such as a bacterial cell line or a
yeast cell line, may alternatively be used. In particular,
it is envisaged that E. coli - derived bacterial strains

10 could be used.
        It is known that some immortalised lymphoid cell lines,
such as myeloma cell lines, in their normal state secrete
isolated Ig light or heavy chains. If such a cell line is
transformed with the vector prepared in step (a) it will

15 not be necessary to carry out step (b) of the process,
provided that the normally secreted chain is complementary
to the variable domain of the Ig chain encoded by the
vector prepared in step (a).
        However, where the immortalised cell line does not

20 secrete or does not secrete a complementary chain, it will
be necessary to carry out step (b). This step may be
carried out by further manipulating the vector produced in
step (a) so that this vector encodes not only the variable
domain of an altered antibody light or heavy chain, but

25 also the complementary variable domain.
        Alternatively, step (b) is carried out by preparing a
second vector which is used to transform the immortalised
cell line. This alternative leads to easier construct
preparation, but may be less preferred than the first

30 alternative in that it may not lead to as efficient
production of antibody.
        In the case where the immortalised cell line secretes a
complementary light or heavy chain, the transformed cell
line may be produced for example by transforming a suitable

35 bacterial cell with the vector and then fusing the

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bacterial cell with the immortalised cell line by
spheroplast fusion. Alternatively, the DNA may be directly
introduced into the immortalised cell line by
electroporation or other suitable method.

5   An antibody is consequently produced in which CDRs of a
variable domain of an antibody chain are homologous with
the corresponding CDRs of an antibody of a first mammalian
species and in which the framework of the variable domain
and the constant domains of the antibody are homologous
10 with the corresponding framework and constant domains of an
antibody of a second, different, mammalian species.
Typically, all three CDRs of the variable domain of a light
or heavy chain are derived from the first species.

        The present process has been applied to obtain an
15 antibody against human CD4 antigen. Accordingly, the
invention also provides an antibody which is capable of
binding to human CD4 antigen, in which the CDRs of the
light chain of the antibody have the amino acid sequences:

        CDR1:   LASEDIYSDLA
20   CDR2:   NTDTLQN
        CDR3:   QQYNNYPWT,

in which the CDRs of the heavy chain of the antibody have
the amino acid sequences:

        CDR1:   NYGMA
25   CDR2:   TISHDGSDTYFRDSVKG
        CDR3:   QGTIAGIRH, and

in which the framework of the variable domain and, if
present, the or each constant domain of each chain are
derived from a mammalian non-rat species.

30   The antibody preferably has the structure of a natural
antibody or a fragment thereof. The antibody may therefore
comprise a complete antibody, a $(Fab')_2$ fragment, a Fab
fragment, a light chain dimer or a heavy chain.

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The antibody may be an IgG such as IgG1, IgG2, IgG3 or
IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may
be a chimaeric antibody of the type described in WO
86/01533.

5   A chimaeric antibody according to WO 86/01533 comprises
an antigen binding region and a non-immunoglobulin region.
The antigen binding region is an antibody light chain
variable domain or heavy chain variable domain. Typically
the chimaeric antibody comprises both light and heavy chain

10   variable domains. The non-immunoglobulin region is fused
at its C-terminus to the antigen binding region. The non-
immunoglobulin region is typically a non-immunoglobulin
protein and may be an enzyme region, a region derived from
a protein having known binding specificity, from a protein

15   toxin or indeed from any protein expressed by a gene. The
two regions of the chimaeric antibody may be connected via
a cleavable linker sequence.

The invention is preferably employed to humanise a CD4
antibody such as a rat or mouse CD4 antibody. The

20   framework and the constant domains of the resulting
antibody are therefore human framework and constant domains
whilst the CDRs of the light and/or heavy chain of the
antibody are rat or mouse CDRs. Preferably all CDRs are
rat or mouse CDRs. The antibody may be a human IgG such as

25   IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat
or mouse CDRs.

Preferably the framework of the antibody heavy chain is
homologous to the corresponding framework of the human
antibody KOL (Schmidt et al, Hoppe-Seyler's Z. Physiol.

30   Chem., 364 713-747, 1983). The sixth residue of
framework 4 in this case is suitably Thr or Pro, preferably
Thr. This residue is the 121st residue in the KOL antibody
heavy chain variable region (Schmidt et al, 1983), and is
identified as residue 108 by Kabat (Kabat et al, "Sequences

35   of proteins of immunological interest", US Dept of Health

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and Human Services, US Government Printing Office, 1987).
Alternatively, the framework of the antibody heavy chain is
homologous to the corresponding framework of the human
antibody NEW (Saul et al, J. Biol.Chem. 253: 585-597,
5   1978). The final residue of framework 1 in this case is
suitably Ser or Thr, preferably Ser. This residue is at
position 30 (Kabat et al, 1987). Preferably the framework
of the antibody light chain is homologous to the variable
domain framework of the protein REI (Epp et al, Eur. J.
10   Biochem., 45, 513-524, 1974).

The framework regions of one or both chains of a CD4
antibody can be reshaped by the present process.
Alternatively, one or both chains of a CD4 antibody may be
reshaped by the procedure described in EP-A-0239400. The
15   procedure of EP-A-0239400 involves replacing CDRs rather
than the replacement of frameworks. The CDRs are grafted
onto a framework derived from a mammalian non-rat species,
typically a human. This may be achieved by
oligonucleotide-directed in vitro mutagenesis of the CDR-
20   encoding regions of an antibody chain, light or heavy, from
a mammalian non-rat species. The oligonucleotides in such
an instance are selected so that the resulting CDR-grafted
antibody has the light chain CDRs 1 to 3 and the heavy
chain CDRs 1 to 3 shown above.

25   The reshaped CD4 antibody can be used to induce
tolerance to an antigen. It can be used to alleviate
autoimmune diseases such as rheumatoid arthritis. It can
be used to prevent graft rejection. Tolerance to a graft
such as an organ graft or a bone marrow transplantation can
30   be achieved. Also, the reshaped CD4 antibody might be used
to alleviate allergies. Tolerance to allergens could be
achieved.

The CD4 antibody may be depleting or non-depleting. A
depleting antibody is an antibody which depletes more than

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50%, for example from 90 to 99%, of target cells _in vivo_.
A non-depleting antibody depletes fewer than 50%, for
example, from 10 to 25% and preferably less than 10% of
target cells _in vivo_. A CD4 antibody may be administered
5 alone or may be co-administered with a non-depleting or
depleting CD8 antibody. The CD4 antibody, depleting or
non-depleting, and CD8 monoclonal antibody, depleting or
non-depleting, may be administered sequentially in any
order or may be administered simultaneously. An additional
10 antibody, drug or protein may be administered before,
during or after administration of the antibodies.

A CD4 antibody and, indeed, a CD8 antibody as
appropriate are given parenterally, for example
intravenously. The antibody may be administered by
15 injection or by infusion. For this purpose the antibody is
formulated in a pharmaceutical composition further
comprising a pharmaceutically acceptable carrier or
diluent. Any appropriate carrier or diluent may be
employed, for example phosphate-buffered saline solution.
20 The amount of non-depleting or depleting CD4 and, if
desired, CD8 antibody administered to a patient depends
upon a variety of factors including the age and weight of a
patient, the condition which is being treated and the
antigen(s) to which it is desired to induce tolerance. In
25 a model mouse system from 1μg to 2mg, preferably from 400μg
to 1mg, of a mAb is administered at any one time. In
humans from 3 to 500mg, for example from 5 to 200mg, of
antibody may be administered at any one time. Many such
doses may be given over a period of several weeks,
30 typically 3 weeks.

A foreign antigen(s) to which it is desired to induce
tolerance can be administered to a host before, during, or
after a course of CD4 antibody (depleting or non-depleting)
and/or CD8 antibody (depleting or non-depleting).
35 Typically, however, the antigen(s) is administered one week

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after commencement of antibody administration, and is
terminated three weeks before the last antibody
administration.

5   Tolerance can therefore be induced to an antigen in a
host by administering non-depleting or depleting CD4 and
CD8 mAbs and, under cover of the mAbs, the antigen. A
patient may be operated on surgically under cover of the
non-depleting or depleting CD4 and CD8 mAbs to be given a
tissue transplant such as an organ graft or a bone marrow
10   transplant. Also, tolerance may be induced to an antigen
already possessed by a subject. Long term specific
tolerance can be induced to a self antigen or antigens in
order to treat autoimmune disease such as multiple
sclerosis or rheumatoid arthritis. The condition of a
15   patient suffering from autoimmune disease can therefore be
alleviated.

     The following Example illustrates the invention. In the
accompanying drawings:

     Figure 1: shows the nucleotide and predicted amino acid
20   sequence of rat CD4 antibody light chain variable region.
The number of the first and last amino acid or nucleotide
in each line is indicated in the left and right margins,
respectively. Base pairs 1-269 (HindIII-PvuII) and 577-620
([BglII/BclI]-BamHI) are part of the vector M13V$_K$PCR3,
25   while base pairs 270-576 are from the PCR product of the
CD4 antibody light chain variable region (V$_L$). CDRs
(boxes) were identified by comparison to known
immunological sequences (Kabat et al, "Sequences of
proteins of immunological interest, US Dept of Health and
30   Human Services, US Government Printing Office, 1987).

     Figure 2: shows the nucleotide and predicted amino acid
sequence of the reshaped CAMPATH-1 antibody light chain
cDNA. The number of the first and last amino acid or
nucleotide in each line is indicated in the left and right
35   margins, respectively. CDRs are identified by boxes.

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Figure 3: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody light chain cDNA CD4V_LREI. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

5

Figure 4: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody heavy chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins,

10 respectively. CDRs are identified by boxes. Base pairs 1-272 (HindIII-PstI) and 603-817 (BstEII-BamHI) are part of the vector M13V_HPCR1, while base pairs 273-602 are from the PCR product of the CD4 antibody heavy chain variable region (V_H).

15

Figure 5: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody heavy chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

20

Figure 6: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Thr[30]. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by

25 boxes.

Figure 7: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Ser[30]. The number of the first and last amino acid or nucleotide in each line is indicated in the left

30 and right margins, respectively. CDRs are identified by boxes.

Figure 8: shows the heavy chain variable (V) region amino acid sequence of the human myeloma protein KOL. CDRs are identified by boxes. This sequence is taken from the

35 Swiss-Prot protein sequence database.

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Figure 9: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro[113]. The number of the first and last amino acid or nucleotide in each line is indicated in the left

5   and right margins, respectively. CDRs are identified by boxes.

Figure 10: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro[113] without immunoglobulin promoter. The

10   number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 11: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region

15   CD4V_HKOL-Thr[113]. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 12: shows the nucleotide and predicted amino acid

20   sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Thr[113] without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

25   Figure 13: shows the results of an ELISA that compares the avidity of YNB46.1.8 and CD4V_HKOL-Thr[113] antibodies. The X-axis indicates the concentration (μg/ml) of YNB46.1.8 (triangles) or CD4V_HKOL-Thr[113] (circles) antibody. The Y-axis indicates the optical density at 492 nanometers.

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EXAMPLE

1. MATERIALS AND METHODS

    Isolation of monoclonal antibody. The rat-derived anti-
human CD4 antibody, clone YNB46.1.8 (IgG_{2b}, kappa light

5 chain serotype), was the result of fusion between a rat
splenocyte and the Lou strain rat myeloma cell line Y3-Ag
1.2.3 (Galfre et al, Nature, 277: 131-133, 1979) and was
selected by its binding to a rat T cell line NB2-6TG stably
transfected with an expression vector containing a

10 complementary DNA (cDNA) encoding the human CD4 antigen
(Madden et al, Cell, 42: 93-104, 1985). Antibody was
purified by high pressure liquid chromatography (HPLC).
    Isolation of Antibody Variable Regions. cDNAs encoding
the V_L and V_H regions of the CD4 antibody were isolated by

15 a polymerase chain reaction (PCR)-based method (Orlandi et
al, PNAS USA, 86: 3833-3837, 1989) with some modifications.
Total RNA was isolated from hybridoma cells by the
guanidine thiocyanate method (Chirgwin et al, Biochemistry,
18: 5294, 1979) and poly(A)$^+$ RNA was isolated by passage

20 of total RNA through and elution from an oligo(dT)-
cellulose column (Aviv and Leder PNAS USA 69: 1408, 1972).
Poly(A)$^+$ RNA was heated at 70°C for 5 minutes and cooled on
ice just prior to use. A 25μl first strand synthesis
reaction consisted of 5μg poly(A)$^+$ RNA, 250 μM each dNTP,

25 50 mM Tris.HCl (pH 8.2 at 42°C), 10 mM $MgCl_2$, 100 mM KCl,
10 mM dithiothreitol, 23 units reverse transcriptase
(Anglian Biotec, Colchester, U.K.), 3.5 pmoles of the V_L
region-specific oligonucleotide primer V_K1FOR [5'-d(GTT AGA
TCT CCA GCT TGG TCC C)] or the V_H region-specific primer

30 $V_H1FOR-B$ [5,-d(TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC)],
and incubated for 5 minutes at 20°C and then 90 minutes at
42°C.

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Subsequent 50 μl PCR amplifications consisted of 5 μl of
the first strand synthesis reaction (unpurified), 500 μM
each dNTP, 67 mM Tris-HCl (pH 8.8 at 25°C), 17 mM
$(NH_4)_2SO_4$, 10 mM $MgCl_2$, 20 μg/ml gelatin, 5 units TAQ DNA

5   polymerase (Koch-Light, Haverhill, U.K.), and 25 pmoles
(each) of primers V_K1FOR and V_K1BACK [5'-d(GAC ATT CAG CTG
ACC CAG TCT CCA)] for the V_L region or V_H1FOR-B and the
mixed primer V_H1BACK [5'-d(AG GT(CG) (CA)A(GA) CTG CAG
(GC)AG TC(TA) GG)] for the V_H region. Reactions were

10 overlayed with mineral oil and subjected to 30 cycles of
1.5 minutes at 95°C (denaturation), 1.5 minutes at 37°C
(V_L) or 50°C (V_H; annealing), and 3 minutes at 72°C
(extension) with a Techne PHC-1 programmable cyclic
reactor. The final cycle contained a 10 minute extension

15 time.

The samples were frozen at -20°C and the mineral oil (a
viscous liquid at -20°C) was removed by aspiration. The
aqueous phases were thawed, and PCR products were purified
by electrophoresis in 2% agarose gels, and then double

20 digested with either PvuII and BglII (V_L) or PstI and
BstEII (V_H) restriction enzymes, and cloned into the PvuII
and BclI restriction sites of the vector M13V_KPCR3 (for V_L
region; Orlandi et al, 1989) or the PstI and BstEII
restriction sites of the vector M13V_HPCR1 (for V_H region).

25 As described in the results, V_L region clones were first
screened by hybridisation to a ^{32}P-labeled oligonucleotide
probe [5'-d(GTT TCA TAA TAT TGG AGA CA)] specific for the
CDR2 of the Y3-Ag 1.2.3 V_L region. V_L region clones not
hybridising to this probe and V_H region clones were

30 sequenced by the dideoxy chain termination method (Sanger
et al, PNAS USA 74: 5463, 1977).

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<u>Reshaped Light Chain Variable Region and Expression
Vector Construct.</u>

The reshaped light chain was constructed by
oligonucleotide-directed <u>in vitro</u> mutagenesis in an M13
vector by priming with three oligonucleotides
simultaneously on a 748 base single-stranded cDNA template
encoding the entire V_L and kappa constant (C_K) regions of
the reshaped CAMPATH-1 antibody (Reichmann et al, Nature
<u>332</u>: 323-327, 1988). The three oligonucleotides [5'-d(AGA
GTG ACC ATC ACC TGT CTA GCA AGT GAG GAC ATT TAC AGT GAT TTA
GCA TGG TAC CAG CAG AAG CCA), 5'-d(CTG CTG ATC TAC AAT ACA
GAT ACC TTG CAA AAT GGT GTG CCA AGC AGA TTC), 5'-d(ATC GCC
ACC TAC TAC TGC CAA CAG TAT AAC AAT TAT CCG TGG ACG TTC GGC
CAA GGG ACC)] were designed to replace each of the three
CDRs in the REI-based human antibody V_L region framework
that is part of the reshaped CAMPATH-1 antibody V_L region
(Reichmann et al, 1988). A clone containing each of the
three mutant oligonucleotides was identified by nucleotide
sequencing and was subcloned into the HindIII site of the
expression vector pHβAPr-1 (Gunning et al, PNAS, <u>84</u>: 4831-
4835, 1987) which also contained a dihydrofolate reductase
gene (Ringold et al, J.Mol.Appl. Genet. <u>1</u>: 165-175, 1981)
driven by a truncated SV40 promoter.

<u>Reshaped Heavy Chain Variable Regions Based on the
Variable Region Framework of the Human Antibody NEW, and
Expression Vector Constructs.</u>

Two versions of the NEW-based reshaped heavy chain were
created, $CD4V_HNEW-Thr^{30}$ and $CD4V_HNEW-Ser^{30}$. The $CD4V_HNEW-
Thr^{30}$ version (Figure 6) encodes a threonine residue at
position 30 while the $CD4V_HNEW-Ser^{30}$ version (Figure 7)
encodes a Ser residue at position 30. As a matter of
convenience, $CD4V_HNEW-Thr^{30}$ was created first by
oligonucleotide-directed <u>in vitro</u> mutagenesis in the vector
M13mp18 by priming with three oligonucleotides

simultaneously on a 1467 base single-stranded cDNA template
(Figure 5) encoding the entire heavy chain of the reshaped
CAMPATH-1 antibody (Reichmann et al, 1988). The three
oligonucleotides [5'-d(TCT GGC TTC ACC TTC ACC AAC TAT GGC

5   ATG GCC TGG GTG AGA CAG CCA CCT), 5'-d(GGT CTT GAG TGG ATT
GGA ACC ATT AGT CAT GAT GGT AGT GAC ACT TAC TTT CGA GAC TCT
GTG AAG GGG AGA GTG),5'-d(GTC TAT TAT TGT GCA AGA CAA GGC
ACT ATA GCT GGT ATA CGT CAC TGG GGT CAA GGC AGC CTC)] were
designed to replace each of the three complementarity

10 determining regions (CDRs) in the NEW-based V_H region that
is part of the reshaped CAMPATH-1 antibody (Reichmann et
al, 1988). A clone (Figure 6) containing each of the three
mutant oligonucleotides was identified by nucleotide
sequencing. CD4V_HNEW-Ser^{30} was created second by

15 oligonucleotide-directed in vitro mutagenesis in the vector
M13mp18 by priming with a single oligonucleotide on the
1458 base single-stranded cDNA template (Figure 6) encoding
CD4V_HNEW-Thr^{30}. The oligonucleotide [5'-d(GCT TCA CCT TCA
GCA ACT ATG GCA T)] was designed to mutate the residue at

20 position 30 from threonine [ACC] to serine [AGC]. A clone
(Figure 7) containing this mutant oligonucleotide was
identified by nucleotide sequencing. Double-stranded forms
of the clones CD4V_HNEW-Thr^{30} and CD4V_HNEW-Ser^{30} were
subcloned as HindIII fragments into the HindIII site of the

25 expression vector pNH316. The vector pNH316 is a modified
version of the vector pHβAPr-1 (Gunning et al, PNAS, 84:
4831-4835, 1987) which was engineered to contain a
neomycin resistance gene driven by a metallothionine
promoter.

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Reshaped Heavy Chain Variable Regions Based on the
Variable Region Framework of the Human Antibody KOL, and
Expression Vector Constructs

Two versions of the KOL-based reshaped heavy chain were
5   created, $CD4V_HKOL$-Thr^{113} and $CD4V_HKOL$-Pro^{113}. The
$CD4V_HKOL$-Thr^{113} version encodes a threonine residue at
position 113 (Figure 11) while the $CD4V_HKOL$-Pro^{113} version
encodes a proline residue at position 113 (Figure 9). As a
matter of convenience, $CD4V_HKOL$-Thr^{113} was created first by
10 oligonucleotide-directed in vitro mutagenesis of single-
stranded DNA template containing the 817 base HindIII-BamHI
fragment encoding the V_H region of the rat CD4 antibody
(Figure 4) cloned into M13mp18 by priming simultaneously
with five oligonucleotides [5'-d(CAC TCC CAG GTC CAA CTG
15 GTG GAG TCT GGT GGA GGC GTG GTG CAG CCT GG), 5'-d(AAG GTC
CCT GAG ACT CTC CTG TTC CTC CTC TGG ATT CAT CTT CAG TAA CTA
TGG CAT G), 5'-d(GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG
TGG), 5'-d(ACT ATC TCC AGA GAT AAT AGC AAA AAC ACC CTA TTC
CTG CAA ATG G), 5'-d(ACA GTC TGA GGC CCG AGG ACA CGG GCG
20 TGT ATT TCT GTG CAA GAC AAG GGA C)] which were designed to
replace the rat framework regions with the human framework
regions of KOL. A clone containing each of the five mutant
oligonucleotides was identified by nucleotide sequencing.
$CD4V_HKOL$-Pro^{113} was created second by oligonucleotide-
25 directed in vitro mutagenesis of single-stranded DNA
template containing the 817 base HindIII-BamHI fragment
encoding $CD4V_HKOL$-Thr^{113} cloned into M13mp18 by priming
with the oligonucleotide [5'-d(TGG GGC AAA GGG ACC CCC GTC
ACC GTC TCC TCA)]. A clone containing this mutant
30 oligonucleotide was identified by nucleotide sequencing.
The immunoglobulin promoters were removed from the
double-stranded DNA forms of clones encoding $CD4V_HKOL$-
Thr^{113} (Figure 11) and $CD4V_HKOL$-Pro^{113} (Figure 9) by
replacing (for both versions) the first 125 bp (HindIII-
35 NcoI) with a HindIII-NcoI oligonucleotide linker fragment

[5'-d(AGC TTT ACA GTT ACT GAG CAC ACA GGA CCT CAC) and its
overlapping complement 5'-d(CAT GGT GAG GTC CTG TGT GCT CAG
TAA CTG TAA)]. The resultant clones, CD4V$_H$KOL-Thr^{113}
(Figure 12) and CD4V$_H$KOL-Pro^{113} (Figure 10), now 731 bp

5 HindIII-BamHI fragments, were separately subcloned into the
HindIII and BamHI cloning sites of the expression vector
pHβAPr-1-gpt (Gunning et al, PNAS USA 76, 1373, 1987) into
which had been cloned the human IgG1 constant region gene
(Bruggemann et al, J.Exp.Med. 166, 1351-1361, 1987) at the

10 BamHI site. Thus, when transfected and expressed as
antibody heavy chains (see below), these reshaped V$_H$
regions are linked to human IgG1 constant regions.

    Fluorescence activated cell sorter (FACS) analysis
    The relative affinities of the reshaped antibodies to

15 bind the CD4 antigen were estimated by FACS analysis. The
CD4-expressing cells used in this analysis were a cloned
rat T cell line NB2-6TG stably transfected with an
expression vector containing a complementary DNA (cDNA)
encoding the human CD4 antigen (Maddon et al, Cell, 42, 93-

20 104, 1985). Cells were stained with the appropriate
reshaped antibody followed by fluorescein-conjugated sheep
anti-human antibodies (Binding Site Ltd., Birmingham, UK).
Control staining (see Table 1) consisted of no antibody
present during the first stage of cell staining. Mean

25 cellular fluorescence was determined with an Ortho FACS.

    Antibody avidity analysis
    The relative avidities of the rat YNB46.1.8 antibody and
the reshaped CD4V$_H$KOL-Thr^{113} antibody were estimated by an
enzyme-linked immunosorbent assay (ELISA). Microtiter

30 plates were coated with soluble recombinant CD4 antigen
(Byrn et al, Nature, 344: 667-670, 1990) at 50 ul/well,
10 ug/ml, and then blocked with 100 ul/well phosphate
buffered saline (PBS) containing 1.0% bovine serum albumin

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(BSA). Antibodies were diluted in PBS containing 0.1% BSA,
and added to wells (50 ul/well) for 45 minutes at room
temperature. Biotinylated $CD4V_HKOL-Thr^{113}$ antibody (10
ul/well; 20 ug/ml final concentration) was then added to

5   each well for an additional 45 minutes. Wells were washed
with PBS containing 0.1% BSA, and then 50 ul streptavidin-
biotinylated horseradish peroxidase complex (Amersham;
Aylesbury, UK) diluted 1:1,000 was added to each well for
30 minutes. Wells were washed with PBS containing 0.1%

10 BSA, and 100 ul substrate (25 mM citric acid, 50 mM
disodium hydrogen phosphate, 0.1% (w/v) o-phenylene
diamine, 0.04% (v/v) 30% hydrogen peroxide) was added to
each well. Reactions were stopped by the addition of 50
ul/well 1.0 M sulfuric acid. Optical densities at 492

15 nanometers (OD_{492}) were determined with an ELISA plate
reader.

   Transfections.
   Dihydrofolate reductase deficient chinese hamster ovary
(CHO^{DHFR-}) cells (10^6/T-75 flask) were cotransfected as

20 described (Wigler et al, PNAS USA 76, 1373, 1979) with 9μg
of heavy chain construct and 1 μg of the light chain
construct. Transfectants were selected in medium
containing 5% dialysed foetal bovine serum for 2 to 3
weeks, and antibody-secreting clones were identified by

25 ELISAs of conditioned media. Antibody was concentrated and
purified by protein-A Sepharose (Trade Mark) column
chromatography.

2. RESULTS

   Cloning of Light and Heavy Chain Variable Region cDNAs.
30   cDNAs encoding the V_L and V_H regions from CD4 antibody-
secreting hybridoma cells were isolated by PCR using
primers which amplify the segment of mRNA encoding the N-

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terminal region through to the J region (Orlandi et al,
1989). V_L and V_H region PCR products were subcloned into
the M13-based vectors M13V$_K$PCR3 and M13V$_H$PCR1,
respectively. Initial nucleotide sequence analysis of
5 random V_L region clones revealed that most of the cDNAs
encoded the V_L region of the light chain expressed by the
Y3-Ag 1.2.3 rat myeloma cell line (Crowe et al, Nucleic
Acid Research, 17: 7992, 1989) that was used as the fusion
partner to generate the anti-CD4 hybridoma. It is likely
10 that the expression of the Y3-Ag 1.2.3 light chain mRNA is
greater than that of the CD4 antibody light chain, or the
Y3-Ag 1.2.3 light chain mRNA is preferentially amplified
during the PCR.
   To maximize the chance of finding CD4 V_L region cDNAs,
15 we first screened all M13 clones by hybridisation to a ^{32}P-
labeled oligonucleotide probe that is complementary to the
CDR 2 of Y3-Ag 1.2.3 (Crowe et al, Nucleic Acid Research,
17: 7992, 1989). Subsequent sequence analysis was
restricted to M13 clones which did not contain sequence
20 complementary to this probe. In this manner, two cDNA
clones from independent PCR amplifications were identified
that encoded identical V_L regions. Nucleotide sequence
analysis of random V_H region PCR products revealed a single
species of V_H region cDNA. Two V_H cDNA clones from
25 independent PCR amplifications were found to contain
identical sequences except that the codon of residue 14
encoded proline [CCT] in one clone while the second clone
encoded leucine [CTT] at the same position.
   According to Kabat et al 1987, 524 of 595 sequenced V_H
30 regions contain a proline residue at this position, while
only 6 contain leucine. We have therefore chosen the
proline-encoding clone for illustration (see below). As
residue 14 lies well within the first V_H framework region
and not in a CDR, it is unlikely to contribute directly to
35 antigen binding, and the ambiguity at this position did not

affect the subsequent reshaping strategy. Thus, we have
not investigated this sequence ambiguity further.

The cDNA sequences and their predicted amino acid
sequences are shown in Figures 1 and 4. As no additional
5 V_L or V_H region-encoding clones were found, it was assumed
that these sequences were derived from the CD4 antibody
genes.

Construction of reshaped antibodies.

Our goal was to investigate the importance of selecting
10 the appropriate human V region framework during reshaping.
Two reshaping strategies were employed.

First reshaping strategy.

In the first strategy, we created a reshaped antibody
that incorporated the CDRs from the rat-derived CD4
15 antibody and the same human V region framework sequences
that we had previously successfully used for the reshaped
CAMPATH-1 antibody, namely an REI-based framework for the
V_L region and an NEW-based framework for the V_H region
(Reichmann et al, 1988). This was accomplished by
20 oligonucleotide-directed in vitro mutagenesis of the six
CDRs of the reshaped CAMPATH-1 antibody light and heavy
chain cDNAs shown in Figures 2 and 5, respectively. The
resultant reshaped CD4 antibody light chain (Figure 3) is
called $CD4V_LREI$. Two versions of the NEW-based reshaped
25 CD4 antibody heavy chain were created: $CD4V_HNEW-Thr^{30}$
(Figure 6) encoding a threonine residue at position 30 (in
framework 1) and $CD4V_HNEW-Ser^{30}$ (Figure 7) encoding a
serine residue at position 30. These two different
versions were created because the successfully reshaped
30 CAMPATH-1 antibody heavy chain bound antigen well whether
position 30 encoded a threonine or serine residue

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(Reichmann et al, 1988), and we chose to test both
possibilities in this case as well.

   Second reshaping strategy

   In the second reshaping strategy, we have reshaped the
5 CD4 antibody V_H region to contain the V_H region framework
sequences of the human antibody KOL. Of all known human
antibody V_H regions, the overall amino acid sequence of the
V_H region of KOL is most homologous to the rat CD4 antibody
V_H region. The V_H regions of the human antibodies KOL and
10 NEW are 66% and 42% homologous to the rat CD4 antibody V_H
region, respectively.

   Two versions of the KOL-based reshaped CD4 antibody
heavy chain V region were created that differ by a single
amino acid residue within the fourth framework region:
15 CD4V_HKOL-Pro[113] (Figure 10) encodes a proline residue at
position 113 and CD4V_HKOL-Thr[113] (Figure 12) encodes a
threonine residue at position 113. CD4V_HKOL-Pro[113] is
"true to form" in that its framework sequences are
identical to those of the KOL antibody heavy chain V region
20 (Figure 8).

   Of all known human antibody V_L regions, the overall
amino acid sequence of the V_L region of the human light
chain NEW is most homologous (67%) to the rat CD4 antibody
V_L region. Thus, the identical reshaped light chain,
25 CD4V_LREI (described above), that was expressed with the
NEW-based reshaped CD4 antibody heavy chains CD4V_HNEW-Thr[30]
and CD4V_HNEW-Ser[30], is also expressed with the KOL-based
reshaped CD4 antibody heavy chains CD4V_HKOL-Pro[113] and
CD4V_HKOL-Thr[113]. This is advantageous because expression
30 of the same reshaped light chain with different reshaped
heavy chains allows for a direct functional comparison of
each reshaped heavy chain.

   To summarise, four different reshaped antibodies were
created. The reshaped light chain of each antibody is

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called $CD4V_LREI$. The reshaped heavy chains of the
antibodies are called $CD4V_HNEW-Thr^{30}$, $CD4V_HNEW-Ser^{30}$,
$CD4V_HKOL-Pro^{113}$, and $CD4V_HKOL-Thr^{113}$, respectively. Each
of the reshaped heavy chains contain the same human IgG1
5 constant region. As each reshaped antibody contains the
same reshaped light chain, the name of a reshaped
antibody's heavy chain shall be used below to refer to the
whole antibody (heavy and light chain combination).

   Relative affinities of the reshaped antibodies
10   The relative affinities of the reshaped antibodies were
approximated by measuring their ability to bind to CD4
antigen-expressing cells at various antibody
concentrations. FACS analysis determined the mean cellular
fluorescence of the stained cells (Table 1).
15   It is clear from this analysis that the reshaped CD4
antibodies bind to CD4 antigen to varying degrees over a
broad concentration range. Consider Experiment 1 of Table
1 first. Comparing $CD4V_HKOL-Thr^{113}$ antibody to $CD4V_HNEW-$
Thr^{30} antibody, it is clear that both antibodies bind CD4^{+}
20 cells when compared to the control, reshaped CAMPATH-1
antibody. However, $CD4V_HKOL-Thr^{113}$ antibody binds CD4^{+}
cells with far greater affinity than $CD4V_HNEW-Thr^{30}$
antibody. The lowest concentration of $CD4V_HKOL-Thr^{113}$
antibody tested (2.5 ug/ml) gave a mean cellular
25 fluorescence nearly equivalent to that of the highest
concentration of $CD4V_HNEW-Thr^{30}$ antibody tested (168
ug/ml). Experiment 2 demonstrates that $CD4V_HNEW-Ser^{30}$
antibody may bind CD4^{+} cells somewhat better than $CD4V_HNEW-$
Thr^{30}. Only 2.5 ug/ml $CD4V_HNEW-Ser^{30}$ antibody is required
30 to give a mean cellular fluorescence nearly equivalent to
10 ug/ml $CD4V_HNEW-Thr^{30}$ antibody. Experiment 3
demonstrates that $CD4V_HKOL-Thr^{113}$ antibody may bind CD4^{+}
cells somewhat better than $CD4V_HKOL-Pro^{113}$ antibody.

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From these assays, it is clear that the KOL-based
reshaped antibodies are far superior to the NEW-based
reshaped antibodies with regards to affinity towards CD4^{+}
cells. Also, there is a lesser difference, if any, between
CD4V$_H$NEW-Thr[30] antibody and CD4V$_H$NEW-Ser[30] antibody, and
likewise between CD4V$_H$KOL-Thr[113] antibody and CD4V$_H$KOL-
Pro[113] antibody. A ranking of these reshaped antibodies
can thus be derived based on their relative affinities for
CD4+ cells:

CD4V$_H$KOL-Thr[113] > CD4V$_H$KOL-Pro[113] >> CD4V$_H$NEW-Ser[30] > CD4V$_H$NEW-Thr[30]

It should be restated that each of the reshaped CD4
antibodies used in the above experiments have the identical
heavy chain constant regions, and are associated with
identical reshaped light chains. Thus observed differences
of binding to CD4+ cells must be due to differences in
their heavy chain V regions.

Relative avidities of the rat YNB46.1.8 antibody and the
reshaped CD4V$_H$KOL-Thr[113] antibody

The relative avidities of the rat YNB46.1.8 antibody and
the reshaped CD4V$_H$KOL-Thr[113] antibody were estimated by
ELISA. In this assay, the ability of each antibody to
inhibit the binding of biotinylated CD4V$_H$KOL-Thr[113]
antibody to soluble recombinant CD4 antigen was determined.
Results of an experiment are shown in Figure 13. The
inhibition of binding of biotinylated CD4V$_H$KOL-Thr[113]
antibody was linear for both the unlabeled CD4V$_H$KOL-Thr[113]
and YNB46.1.8 antibodies near the optical density of 0.3.
The concentrations of CD4V$_H$KOL-Thr[113] and YNB46.1.8
antibodies that give an optical density of 0.3 are 28.7 and
1.56 ug/ml, respectively. Thus the avidity of the
YNB46.1.8 antibody can be estimated to be 28.7/1.56 or
about 18 times better than that of CD4V$_H$KOL-Thr[113]
antibody. It should be noted that this experiment only
provides a rough approximation of relative avidities, not

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affinities. The rat YNB46.1.8 antibody contains a
different constant region than that of the $CD4V_HKOL-Thr^{113}$
antibody, and this could affect how well the antibodies
bind CD4 antigen, irrespective of their actual affinities
5   for CD4 antigen. The actual affinity of the reshaped
antibodies for CD4 antigen may be greater, lesser, or the
same as the YNB46.1.8 antibody. The other reshaped
antibodies $CD4V_HKOL-Pro^{113}$, $CD4V_HNEW-Ser^{30}$, and $CD4V_HNEW-$
Thr^{30} have not yet been tested in this assay.

Table 1.   Mean cellular fluorescence of CD4$^+$ cells
              stained with reshaped antibodies

Reshaped Antibody	Concentration (μg/ml)	Mean cellular Fluorescence
Experiment 1.		
CD4V$_H$KOL-Thr^{113}	113	578.0
CD4V$_H$KOL-Thr^{113}	40	549.0
CD4V$_H$KOL-Thr^{113}	10	301.9
CD4V$_H$KOL-Thr^{113}	2.5	100.5
CD4V$_H$NEW-Thr^{30}	168	97.0
CD4V$_H$NEW-Thr^{30}	40	40.4
CD4V$_H$NEW-Thr^{30}	10	18.7
CD4V$_H$NEW-Thr^{30}	2.5	10.9
CAMPATH-1	100	11.6
CAMPATH-1	40	9.4
CAMPATH-1	10	9.0
CAMPATH-1	2.5	8.6
CONTROL	----	9.0
Experiment 2.		
CD4V$_H$NEW-Thr^{30}	168	151.3
CD4V$_H$NEW-Thr^{30}	40	81.5
CD4V$_H$NEW-Thr^{30}	10	51.0
CD4V$_H$NEW-Thr^{30}	2.5	39.3
CD4V$_H$NEW-Ser^{30}	160	260.2
CD4V$_H$NEW-Ser^{30}	40	123.5
CD4V$_H$NEW-Ser^{30}	10	68.6
CD4V$_H$NEW-Ser^{30}	2.5	49.2
CONTROL	----	35.8
Experiment 3.		
CD4V$_H$KOL-Pro^{113}	100	594.9
CD4V$_H$KOL-Pro^{113}	40	372.0
CD4V$_H$KOL-Pro^{113}	10	137.7
CD4V$_H$KOL-Pro^{113}	2.5	48.9
CD4V$_H$KOL-Thr^{113}	100	696.7
CD4V$_H$KOL-Thr^{113}	40	631.5
CD4V$_H$KOL-Thr^{113}	10	304.1
CD4V$_H$KOL-Thr^{113}	2.5	104.0
CONTROL	----	12.3

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CLAIMS

1.   A process for the preparation of an antibody
chain in which the complementarity determining regions
(CDRs) of the variable domain of the antibody chain are
derived from a first mammalian species and the framework of
the variable domain and, if present, the or each constant
domain of the antibody chain are derived from a second
different mammalian species, which process comprises:
          (i)   mutating the framework-encoding regions
of DNA encoding a variable domain of an antibody chain of
the said first species such that the mutated framework-
encoding regions encode the said framework derived from the
said second species; and
          (ii) expressing the said antibody chain
utilising the mutated DNA from step (i).

2.   A process according to claim 1, wherein the
framework-encoding regions of DNA encoding the variable
domain of an antibody heavy chain are mutated in step (i).

3.   A process according to claim 1 or 2, wherein
the framework-encoding regions of DNA encoding the variable
domain of an antibody light chain are mutated in step (i).

4.   A process according to any one of the
preceding claims, wherein the said first species is rat or
mouse.

5.   A process according to any one of the
preceding claims, wherein the said second species is human.

6.   A process according to any one of the
preceding claims, comprising:

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        (a)   determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;

5   (b)   determining the antibody framework to which the framework of the said domain is to be altered;

        (c)   mutating the framework-encoding regions of DNA encoding the said variable domain such that mutated framework-encoding regions encode the framework determined upon in step (b).

10   (d)   linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and

        (e)   introducing the expression vector into a compatible host cell and culturing the host cell under such

15 conditions that antibody chain is expressed.

        7.   A process according to claim 6, in which about the most homologous framework of an antibody chain of a different species is selected in step (b) as the framework to which the framework of the said variable domain is to be

20 altered.

        8.   A process according to any one of the preceding claims, wherein the antibody of the said first species is a CD4 antibody.

        9.   A process according to any one of the

25 preceding claims, wherein the said antibody chain is co-expressed with a complementary antibody chain and antibody comprising the said two chains is recovered.

        10.   An antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of

30 the antibody have the amino acid sequences:

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            CDR1:       LASEDIYSDLA
            CDR2:       NTDTLQN
            CDR3:       QQYNNYPWT
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in which the CDRs of the heavy chain of the antibody have
5   the amino acid sequences:
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            CDR1:       NYGMA
            CDR2:       TISHDGSDTYFRDSVKG
            CDR3:       QGTIAGIRH, and
```
in which the framework of the variable domain and, if
10 present, the or each constant domain of each chain are
derived from a mammalian non-rat species.

        11.   An antibody according to claim 10, in which
    the mammalian non-rat species is human.

        12.   An antibody according to claim 11, in which
15 the variable domain framework of the heavy chain is
    homologous to the heavy chain variable domain framework of
    the protein KOL.

        13.   An antibody according to claim 12, in which
    the heavy chain variable region has the amino acid sequence
20 shown in the upper line in Figure 10 or 12.

        14.   An antibody according to claim 11, in which
    the variable domain framework of the heavy chain is
    homologous to the heavy chain variable domain framework of
    the protein NEW.

25   15.   An antibody according to claim 14, in which
    the heavy chain variable region has the amino acid sequence
    shown in the upper line of Figure 6 or 7.

        16.   An antibody according to any one of claims 11
    to 15, in which the variable domain framework of the light

- 35 -

chain is homologous to the variable domain framework of the
protein REI.

17. An antibody according to claim 16, in which
the light chain has the amino acid sequence shown in the
5 upper line of Figure 3.

18. A pharmaceutical composition comprising a
pharmaceutically acceptable carrier or diluent and, as
active ingredient, an antibody as claimed in any one of
claims 10 to 17.

FIG.1

```
        HindIII
  1     AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACC      59

 60     ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTGAGCACACAGGACCTCA     119

-19      M   G   W   S   C   I   I   L   F   L   V   A   T   A   T        -5
120     CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGTGCA     179

180     CAGTAGCAGGCTTGAGGTCTGGACATATATGGGTGACAATGACATCCACTTTGCCTTT      239

 -4      G   V   H   S   D   I   Q   L   T   Q   S   P   V   S   L   S   A   13
240     CTCTCCACAGGTGTCCACTCCGACATCCAGCTGACCCAGTCTCCAGTTTCCCTGTCTGCA     299

 14      S   L   G   E   T   V   N   I   E   C | L   A   S   E   D   I   Y   S   D   L   33
                                                CDR1
300     TCTCTGGGAGAAACTGTCAACATCGAATGTCTAGCAAGTGAGGACATTTACAGTGATTTA     359
```

FIG.1 (contd.)

```
                                                      CDR 2
34  A   W   Y   Q   Q   K   P   G   K   S   P   Q   L   L   I   Y │N   T   D   T│         53
360 GCAATGGTATCAGCAGAAGCCAGGGAAATCTCCTCAACTCCTGATCTATAATACAGATACC                419

54 │L   Q   N│ G   V   P   S   R   F   S   G   S   G   S   G   T   Q   Y   S   L   73
420 TTGCAAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCGGGCACACAGTATTCTCTA                479

                                                      CDR 3
74  K   I   N   S   L   Q   S   E   D   V   A   T   Y   F   C │Q   Q   Y   N   N│   93
480 AAAATAAACAGCCTGCAATCTGAAGATGTCGCGACTTATTTCTGTCAACAATATAACAAT                539

94 │Y   P   W   T│ F   G   G   G   T   K   L   E   I   K   R                      108
540 TATCCGTGGACGTTCGGTGGAGGGACCAAGCTGGAGATCAAACGTGAGTAGAATTTAAAC                599

                    BamHI
600 TTTGCTTCCTCAGTTGGATCC                                                        620
```

FIG. 2

```
                              M   G   W   S   C   I
-19                                                              -14
 1   AAGCTTGGCTCTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATC    58
     HindIII
                                                        CDR 1
     I   L   F   L   V   A   A   T   G   V   H   S   D   I   Q   M   T   Q   S
-13                                                              7
59   ATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCGACATCCAGATGACCCAGAGC   118

     P   S   S   L   S   A   S   V   G   D   R   V   T   I   T   C  |K   A   S   Q|
 8                                                               27
119  CCAAGCAGCCTGAGCGCCAGCGTGGGTGACAGAGTGACCATCACCTGTAAAGCAAGTCAG   178

    |N   I   D   K   Y   L   N|  W   Y   Q   Q   K   P   G   K   A   P   K   L   L
28                                                               47
      CDR 2
179  AATATTGACAAATACTTAAACTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTCCTG   238

     I   Y  |N   T   N   L   Q   T|  G   V   P   S   R   F   S   G   S   G   S
48                                                               67
         CDR 2
239  ATCTACAATACAAACAATTTGCAAACGGGTGTGCCAAGCAGATTCAGCGGTAGCGGTAGC   298

     G   T   D   F   F   T   I   S   S   L   Q   P   E   D   I   A   T   Y   Y
68                                                               87
299  GGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACATCGCCACCTACTAC   358
```

FIG. 2(contd.)

```
        ┌─ CDR 3 ─────────┐
        C  L  Q  H  I  S  R  P  R  T  F  G  Q  G  T  K  V  E  I  K      107
88
359     TGCTTGCAGCATATAAGTAGGCCGCGCACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA    418

        R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S      127
108
419     CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTGAAATCT     478

        G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q      147
128
479     GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG    538

        W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D      167
148
539     TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC    598

        S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E      187
168
599     AGCAAGGACAGCACCTACAGCCTCAGCGCTGCCACCCTGAGCGCTGAGCAAAGCAGACTACGAG    658

        K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K      207
188
659     AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG

        S  F  N  R  G  E  C  Trm  HindIII                                214
208
719     AGCTTCAACAGGGGAGAGTGTTAGAAGCTT                                    748
```

FIG. 3

```
-19                      M   G   W   S   C   I                              -14
  1   HindIII                                                                58
      AAGCTTGGCTCTACACTTACTGAGCACCAGGACCTCACCATGGGATGGAGCTGTATC

-13   I   L   F   L   V   A   T   A   T   G   V   H   S   D   I   Q   M   T   Q   S    7
 59                                                                        118
      ATCCTCTTCTTGGTAGCAACAGTCACAGTGTCCACTCCGACATCCAGATGACCCAGAGC
                                                             CDR 1
  8   P   S   S   L   S   A   S   V   G   D   R   V   T   I   T   C  [L   A   S   E]  27
119                                                                        178
      CCAAGCAGCCTGAGCGCCAGCGTGGGTGACAGAGTGACCATCACCTGTCTAGCAAGTGAG

 28  [D   I   Y   S   D   L   A]  W   Y   Q   Q   K   P   G   K   A   P   K   L   L   47
179        CDR 2                                                           238
      GACATTTACAGTGATTTAGCATGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTG

 48   I   Y  [N   T   D   T   L   Q   N]  G   V   P   S   R   F   S   G   S   G   S   67
239                                                                        298
      ATCTACAATACAGATACCTTGCAAAATGGTGTGCCAAGCAGATTCAGCGGTAGCGGTAGC

 68   G   T   D   F   T   F   T   I   S   S   L   Q   P   E   D   I   A   T   Y   Y   87
299                                                                        358
      GGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACATCGCCACCTACTAC

 88   C  [Q   Q   Y   N   N   Y   P   W   T]  F   G   Q   G   T   K   V   E   I   K  107
359        CDR 3                                                           418
      TGCCAACAGTATAACAATTATCCGTGGACTTTCGGCCAAGGGACCAAGGTGGAAATCAAA
```

FIG. 3 (contd)

```
108  R   T   V   A   A   P   S   V   F   I   F   P   P   S   D   E   Q   L   K   S   127
419  CGAACTGTGGCTGCCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT  478

128  G   T   A   S   V   V   C   L   L   N   N   F   Y   P   R   E   A   K   V   Q   147
479  GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCATCCCAGAGAGGCCAAAGTACAG  538

148  W   K   V   D   N   A   L   Q   S   G   N   S   Q   E   S   V   T   E   Q   D   167
539  TGGAAGGTGGATAACGCCCTCCAATCGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC  598

168  S   K   D   S   T   Y   S   L   S   S   T   L   T   L   S   K   A   D   Y   E   187
599  AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG  658

188  K   H   K   V   Y   A   C   E   V   T   H   Q   G   L   S   S   P   V   T   K   207
659  AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG  718

208  S   F   N   R   G   E   C  Trm  HirdIII                                 214
719  AGCTTCAACAGGGGAGAGTGTTAGAAGCTT                                         748
```

FIG.4

```
        HindIII
1    AAGCTTATGAATATGCAAATCCTCTGAATCTACACATGGTAAATATAGGTTTGTCTATACC   59

60   ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTCAGCACACAGGACCTCA   119

-19         M  G  W  S  C  I  I  L  F  L  V  A  T  A  T
120  CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCA   179

180  CAGTAGCAGGCTTGAGGTCTGACACATATATGGGTGACAATGACATCCACTTTGCCTTT   239

-4          G  V  H  S  Q  V  Q  L  Q  E  S  G  G  G  L  V  Q
240  CTCTCCACAGTGTCCACTCCCAGGTCCAACTGCAGGAGTCTGGTGGAGGCTTAGTGCAG   299

                                              CDR 1
14          P  G  R  S  L  K  L  S  C  A  A  S  G  L  T  F  S | N  Y  G |
300  CCTGGAAGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGACTCACTTTCAGTAACTATGGC   359

                                                    CDR 2
34          M  A  W  V  R  Q  A  P  T  K  G  L  E  W  V  A | T  I  S  H |
360  ATGGCCTGGGTCCGCCAGGCTCCAACGAAGGGGCTGGAGTGGGTCGCAACCATTAGTCAT   419
```

FIG. 4 (contd.)

```
54      D  G  S  D  T  Y  F  R  D  S  V  K  G | R  F  T  I  S  R  D      73
420   GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT    479

74      N  G  K  S  T  L  Y  L  Q  M  D  S  L  R  S  E  D  T  A  T      93
480   AATGGAAAAGCACCCTATACCTGCAAATGACAGTCTGAGGTCTGAGGACACGGCCACT     539

94      Y  Y  C  A  R | Q  G  T  I  A  G  I  R  H | W  G  Q  G  T  T     113
540   TATTACTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAGGGACCACG    599

114     V  T  V  S  S                                                  118
600   GTCACCGTCTCCTCCCAGGTGAGTCCTTACAACCTCTCTCTTCTATTCAGCTTAAATAGATT  659

660   TTACTGCATTGTTGGGGGGGAAATGTGTTGTATCTGAATTTCAGGTCATGAAGGACTAGG   719

720   GACACCTTGGGAGTCAGAAAAGGGTCATTGGGAGCCCCGGGCTGATGCAGCAGACATCCTC  779

                         BamHI
780   AGCTCCCAGAGACTTCATGGCCAGAGATTTATAGGGATCC                       817
```

FIG.5

```
                                     M   G   W   S   C   I   I   L        -12
-19  HindIII
  1  AAGCTTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC          59

-11  F   L   V   A   T   A   T   G   V   H   S   Q   V   Q   L   Q   E   S   G   P       9
 60  TTCTTGGTAGCAACAGCTACAGTGTCCCACTCCCAGGTCCAACTCGAGGACAGCGGTCCA        119

 10  G   L   V   R   P   S   Q   T   L   S   L   T   C   T   V   S   G   F   T   F      29
120  GGTCTTGTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC       179

                     CDR 1
 30  T  [D   F   Y   M   N]  W   V   R   Q   P   P   G   R   G   L   E   W   I   G      49
180  ACCGATTTCTACATGAACTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGA       239

                                 CDR 2
 50 [F   I   R   D   K   A   K   G   Y   T   T   E   Y   N   P   S   V   K   G]  R      69
240  TTTATTAGAGACAAAGCTAAAGGTTACACAACAGAGTACAATCCATCTGTGAAGGGGAGA       299
```

FIG. 5 (cont'd.)

```
70  V  T  M  L  V  D  T  S  K  N  Q  F  S  L  R  L  S  S  V  T              89
300 GTGACAAATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACA        359

                                           ┌─────CDR 3─────┐
90  A  A  D  T  A  V  Y  Y  C  A  R  E  G  H  T  A  A  P  F  D             109
360 GCCGCCGACACCGGGTCTATTATTGTGCAAGAGAGGGCCACACTGCTGCTCCTTTGAT           419

110 Y  W  G  Q  G  S  L  V  T  V  S  S  A  S  T  K  G  P  S  V             129
420 TACTGGGGTCAAGGCAGCCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTC       479

130 F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L             149
480 TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG        539

150 V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S             169
540 GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC        599
```

FIG.5 (contd.)

```
170                G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  S  V   189
600  GGCGTGCACACTTCCCGGCTGTCCTACAGTCCTCAGGACTTCTACTCCCTCAGCAGCGTG  659

190                V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K   209
660  GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG  719

210                P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T   229
720  CCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA  779

230                C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P   249
780  TGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCA  839

250                K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D   269
840  AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC  899
```

FIG. 5 (contd.)

```
270  V   S   H   E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   289
900  GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT  959

290  N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   309
960  AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC  1019

310  L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   329
1020 CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC 1079

330  K   A   L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   349
1080 AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA 1139

350  P   Q   V   Y   T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   369
1140 CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTG 1199
```

FIG. 5 (contd.)

```
370  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G        389
1200 ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG    1259

390  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F        409
1260 CAGCCCGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGGCTCCTTCTTC   1319

410  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C        429
1320 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC    1379

430  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P        448
1380 TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG    1439

449  G  K  Trm          HindIII                                       450
1440 GGTAAATGAGTGCGACGGCCCCAAGCTT                                     1467
```

FIG. 6

					M	G	W	S	C	I	I	L	-12

```
-19  HindIII
 1   AAGCTTTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC    59

          F  L  V  A  T  A  T  G  V  H  S  Q  V  Q  L  Q  E  S  G  P  P      9
-11  TTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGGAGAGCGGTCCA   119
 60

          G  L  V  R  P  S  Q  T  L  S  L  T  C  T  V  S  G  F  T  F      29
 10  GGTCTTGTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC   179
120          CDR 1

          T │N  Y  G  M  A│ W  V  R  Q  P  P  G  R  G  L  E  W  I  G      49
 30  ACCAACTATGGCATGGCCTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGA   239
180                         CDR 2

          T │I  S  H  D  G  S  D  T  Y  F  R  D  S  V  K  G│ R  V  T      69
 50  ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAAGGGGAGAGTGACA   299
240
```

FIG. 6 (contd.)

```
 70  M   L   V   D   T   S   K   N   Q   F   S   L   R   L   S   S   V   T   A   A    89
300  ATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC                   359

                                      CDR 3
 90  D   T   A   V   Y   Y   C   A   R  | Q   G   T   I   A   G   I   R   H | W   G   109
360  GACACCGCGGTCTATTATTGTGCAAGACAAGGCACTATAGCTGGTATACGTCACTGGGGT                   419

110  Q   G   S   L   V   T   V   S   S   A   S   T   K   G   P   S   V   F   P   L   129
420  CAAGGCAGCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG                   479

130  A   P   S   S   K   S   T   S   G   G   T   A   A   L   G   C   L   V   K   D   149
480  GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC                   539

150  Y   F   P   E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H   169
540  TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC                   599
```

FIG. 6 (contd.)

```
170  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V           189
600  ACCTTCCCGGCCTGTCCTACAGTCCTCAGGACTCCTACTCCCTCAGCAGCGTGGTGACCGTG       659

190  P  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N              209
660  CCCTCCAGCAGCTTGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC          719

210  T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T  C  P  P           229
720  ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG         779

230  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K           249
780  TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG         839

250  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H           269
840  GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC         899
```

FIG. 6 (contd.)

```
270                                                                              289
E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGGGTGGAGGTGCATAATGCCAAG
900                                                                              959

290                                                                              309
T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V
ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC
960                                                                             1019

310                                                                              329
L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L
CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC
1020                                                                            1079

330                                                                              349
P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V
CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG
1080                                                                            1139

350                                                                              369
Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L
TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG
1140                                                                            1199
```

FIG. 6 (contd.)

```
370   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E    389
1200  GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG   1259

390   N   N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S    409
1260  AACAACTACAAGACCACGCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC   1319

410   K   L   T   V   D   K   S   R   W   Q   Q   G   N   V   F   S   C   S   V   M    429
1320  AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGTCTTCTTCATGCTCCGTGATG   1379

430   H   E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P   G   K  Trm   448
1380  CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA   1439

                            HindIII
1440  GTGCGGACGGCCCCAAGCTT                                            1458
```

FIG. 7

```
-19                                          M   G   W   S   C   I   I   L    -12
  1  HindIII
     AAGCTTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC          59

-11   F   L   V   A   T   A   T   G   V   H   S   Q   V   Q   L   Q   E   S   G   P     9
 60  TTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGGAGAGCGGTCCA        119

 10   G   L   V   R   P   S   Q   T   L   S   L   T   C   T   V   S   G   F   T   F    29
120  GGTCTTGTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC        179
                                            CDR 1

 30   S  [N   Y   G   M   A]  W   V   R   Q   P   P   G   R   G   L   E   W   I   G    49
180  AGCAACTATGGCATGGCCTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGA        239
                                            CDR 2

 50  [T   I   S   H   D   G   S   D   T   Y   F   R   D   S   V   K   G]  R   V   T    69
240  ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAAGGGGAGAGTGACA        299
```

FIG. 7 (contd.)

```
70    M   L   V   D   T   S   K   N   Q   F   S   L   R   L   S   S   V   T   A   A                          89
300   ATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTGAGCAGCGTGACAGCCGCC                                           359

                                                        CDR 3
90    D   T   A   V   Y   Y   C   A   R | Q   G   T   I   A   G   I   R | H   W   G                          109
360   GACACCGCGGTCTATTATTGTGCAAGACAAGGCACTATAGCTGGTATACGTCACTGGGGT                                           419

110   Q   G   S   L   V   T   V   S   S   A   S   T   K   G   P   S   V   F   P   P   L                      129
420   CAAGGCAGCCTGGTCACAGTCTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG                                         479

130   A   P   S   S   K   S   T   S   G   G   T   A   A   L   G   C   L   V   K   D                          149
480   GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC                                           539

150   Y   F   P   E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H                          169
540   TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC                                           599
```

FIG. 7 (contd.)

```
170  T   F   P   A   V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T   V   189
600  ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG   659

190  P   S   S   L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S   N   209
660  CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC   719

210  T   K   V   D   K   K   V   E   P   K   S   C   D   K   T   H   T   C   P   P   229
720  ACCAAGGTGGACAAGAAAGTTGACCCCAAATCTTGTGACAAAACTCACACATGCCCACCG   779

230  C   P   A   P   E   L   L   G   G   P   S   V   F   L   F   P   P   K   P   K   249
780  TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG   839

250  D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S   H   269
840  GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC   899
```

FIG. 7 (contd.)

```
270                E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K            289
900   GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG            959

290                T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V            309
960   ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC           1019

310                L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L            329
1020  CTCCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC           1079

330                P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V            349
1080  CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG           1139

350                Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L            369
1140  TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG           1199
```

FIG. 7 (contd.)

```
370   V  K  G  F  Y  P  S  D  I  A  V  E  W  E  E  S  N  G  Q  P  E    389
1200  GTCAAAGGCTTCTATCCCAGCGACATCCCGTGGGAGTGGGAGAGCCAATGGGCAGCCCGAG   1259

390   N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S    409
1260  AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC   1319

410   K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M    429
1320  AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAAACGTCTTCTCATGCTCCGTCATG   1379

430   H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K  Trm   448
1380  CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA   1439

                    HindIII
1440  GTGCGACGGCCCCAAGCTT                                            1458
```

FIG.8

```
  1   Q V Q L V E S G G G V V Q                              13

 14   P G R S L R L S C A A S G F I F S S Y A                33
                                     CDR 1  └───┘

 34   M Y W V R Q A P G K G L E W V A A I W D                53
     └─┘ CDR 1                      CDR 2  └─────┘

 54   D G S D Q H Y A D S V K G R F T I S R D                73

 74   N S K N T L F L Q M D S L R P E D T G V                93

 94   Y F C A R D G G H G F C S S A S C F G P               113
              CDR 3  └─────────────────────┘

114   D Y W G Q G T P V T V S S                             126
     └─┘
```

FIG. 9

HindIII
```
1    AAGCTTATGAATATGCAAATCCTCTGAATCTACAGTGGTAAAATAGGTTTGTCTATACC          59

60   ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTCACACAGGACCTCA          119

      M  G  W  S  C  I  I  L  F  L  V  A  T  A  T
-19                                                                       -5
120  CCATGGGATGGAGCTGTATCATCCTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCA          179

180  CAGTAGCAGGCTTGAGGTCTGGACATATATGGGTGACAATGACATCCACTTTGCCTTT          239

      G  V  H  S  Q  V  Q  L  V  E  S  G  G  G  V  V  Q
-4                                                                        13
240  CTCTCCACAGGTGTCCACTCCCAGGTCCAACTGGTGGAGTCTGGCGTGGTGAGCCTGTGGTGCAG   299

                                                    CDR1
      P  G  R  S  L  R  L  S  C  S  S  S  G  F  I  F  S  | N  Y  G |
14                                                                        33
300  CCTGGAAGGTCCCTGAGACTCTCCTGTTCCTCCTCTGGATTCATCTTCAGTAACTATGGC        359

                                                    CDR2
     | M  A | W  V  R  Q  A  P  G  K  G  L  E  W  V  A  | T  I  S  H |
34                                                                        53
360  ATGGCCTGGGTCCCCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTCGCAACCATTAGTCAT        419
```

FIG. 9 (contd.)

```
54   D   G   S   D   T   Y   F   R   D   S   V   K  [G   R   F   T   I   S   R   D    73
420  GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT     479

74   N   S   K   N   T   L   F   L   Q   M   D   S   L   R   P   E   D   T   G   V    93
480  AATAGCAAAAACACCCTATTCCTGCAAATGGACAGTCTGAGGCCCCAGGACACGGGGGCGTG     539
                              CDR 3
94   Y   F   C   A   R [Q   G   T   I   A   G   I   R   H   W   G   Q   G   T   P    113
540  TATTTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCCAAGGGACCCCC     599

114  V   T   V   S   S                                                             118
600  GTCACCGTCTCCTCCAGGTGAGTCCTTACAACCTCTCTTCTATTCAGCTTAAATAGATT     659

660  TTACTGCATTGTTGGGGGGAAATGTGTATCTGAATTCAGGTCATGAAGGACTAGG        719

720  GACACCTTGGGAGTCAGAAAGGGTCATTGGGAGCCCGGGCTCATGCAGACAGACATCCTC     779

                           BanHI
780  AGCTCCCAGACTTCATGGCCAGAGATTTATAGGGATCC                         817
```

FIG.10

```
                   M  G  W  S  C  I  I  L  F     -11
-19                                               60
 1   AAGCTTTACAGTTACTCAGCACACGGACCTCACCATGGGATGGAGCTGTATCATCCTCT
     HindIII

     L  V  A  T  A  T                            -5
-10                                              120
61   TCTTGGTAGCAACAGCTACAGGTAAGGGCTCACAGTAGCAGGCTTGAGGTCTGACATA

                        G  V  H  S  Q  V           2
-4                                               180
121  TATATGGGTGACAATGACATCCACTTTGCCTTTCTCCACAGTGTCCACTCCCAGGTC

     Q  L  V  E  S  G  G  G  V  V  Q  P  P  G  R  S  L  R  L  S  C    22
 3                                               240
181  CAACTGGTGGAGTCTGGTGGAGGCGTGGTGCAGCCTGGAAGGTCCCTGAGACTCTCCTGT

     S  S  S  G  F  I  F  S  [N  Y  G  M  A]  W  V  R  Q  A  P  G     42
23            CDR 1                              300
241  TCCTCCTCTGGATTCATCTTCAGTAACTATGGCATGGCCTGGGTCCGCCAGGCTCCAGGC

     K  G  L  E  W  V  A  [T  I  S  H  D  G  S  D  T  Y  F  R  D]     62
43                     CDR 2                      360
301  AAGGGGCTGGAGTGGGTCGCAACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGAC
```

FIG.10 (contd.)

```
            S   V   K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   F   L   Q
63                                                                          CDR 3
361  TCCGTGAAGGGCCGATTCACTATCTCCAGAGATAATAGCAAAAACACCCTATTCCTGCAA                          82

                                                                       ┌─────────┐
            M   D   S   L   R   P   E   D   T   G   V   Y   F   C   A   R │ Q   G   T   I │
83                                                                     └─────────┘       420
421  ATGGACAGTCTGAGGCCCGAGGACACGGGCGTGTATTTCTGTGCAAGACAAGGGACTATA                          102

     ┌─────────┐                                                                         480
     │ A   G   I   R   H │ W   G   Q   G   T   P   V   T   V   S   S
103  └─────────┘                                                                         122
481  GCAGGTATACGTCACTGGGGCCAAGGGACCCCCGTCACCGTCTCCTCAGTGAGTCCTTA                           540

541  CAACCTCTCTTCTATTCAGCTTAAATAGATTTTACTCGCATTCGTTGGGGGGAAATGT                            600

601  GTGTATCTGAATTTCAGGTCATCGAAGGACTAGGGACACCTTGGGAGTCAGAAAGGGTCAT                          660

661  TGGGAGCCCGGGCTGATGCAGACAGACATCCTCAGCTCCCAGACTTCATGCCCAGAGATT                           720

                BamHI
721  TATAGGGATCC                                                                          731
```

FIG.11

```
HindIII
  1   AAGCTTATGAATATGCAAATCCTCTGAATCTACACATGGTAAATATAGGTTTGTCTATACC    59
 60   ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACACGTTACTCAGCACACAGGACCTCA   119

-19         M   G   W   S   C   I   I   L   F   L   V   A   T   A   T
120   CCATGGGATGGAGCTGTATCATCCCTTCTTGCTAGCAACAGCTACAGGTAAGGGGCTCA     -5
                                                                    179

-4          G   V   H   S   Q   V   Q   L   V   E   S   G   G   G   V   V   Q
240   CTCTCCACAGGTGTCCACTCCCAGTCCAACTGGTGAGTCTGGTGGAGGCGTGGTGCAG      13
                                                                    299

                                                        CDR 1
 14    P   G   R   S   L   R   L   S   C   S   S   S   G   F   I   F   S  │ N   Y   G │
300   CCTGGAAGGTCCCTGAGACTCTCCTGTTCCCCTCTGGATTCATCTTCAGTAACTATGGC     33
                                                                    359

                                                        CDR 2
 34   │ M   A │ W   V   R   Q   A   P   G   K   G   L   E   W   V   A  │ T   I   S   H │
360   ATGGCCTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTCGCAACCATTAGTCAT    53
                                                                    419
```

180   CACTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTTT   239

FIG.11 (contd.)

```
54  D   G   S   D   T   Y   F   R   D   S   V   K   G   R   F   T   I   S   R   D    73
420 GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT   479

74  N   S   K   N   T   L   F   L   Q   M   D   S   L   R   P   E   D   T   G   V    93
                              CDR 3
480 AATAGCAAAAACACCCTATTCCTGCAAATGGACAGTCTGAGGCCCGAGGACACGGGCGTG   539

94  Y   F   C   A   R   Q   G   T   I   A   G   I   R   H   W   G   Q   G   T   113
540 TATTTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAGGGACCACG   599

114 V   T   V   S   S   118
600 GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTATTCAGCTTAAATAGATT   659

660 TTACTGCATTTGTTGGGGGGAAATGTGTGTATCTGAATTTCAGGTCATGAAGGACTAGG   719

720 GACACCTTGGGAGTCAGAAAGGGTCATTGGAGCCCGGGCTGATGCGACAGACAGACATCCTC   779

                              BanHI
780 AGCTCCCAGACTTCATGGCCAGAGATTTATAGGGATCC   817
```

FIG. 12

```
-19                          M   G   W   S   C   I   I   L   F      -11
 1   AAGCTTACAGTTACTCAGCACACAGGACCTCCACCATGGGATGGAGCTGTATCATCCTCT    60
     HindIII

-10    L   V   A   T   A   T                                        -5
61   TCTTGGTAGCAACAGCTACAGGTAAGGGCTCACAGTAGCAGGCTTGAGGTCTGGACATA   120

-4                                   G   V   H   S   Q   V            2
121  TATATGGGTGACAAATGACATCCACTTTGCCTTTCTCCACAGTGTCCACTCCCAGGTC    180

 3   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L   R   L   S   C    22
181  CAACTCGTGGAGTCTGGTGGAGGCGTGGTGCAGCCTGGCAGGTCCCTGAGACTCTCCTGT  240

23   S   S   S   G   F   I   F   S  [N   Y   G   M   A]  W   V   R   Q   A   P   G    42
                                    CDR 1
241  TCCTCCCTCTGATTCATCTTCAGTAACTATGGCATGGCCTGGGTCCGCCAGGCTCCAGGC  300

43   K   G   L   E   W   V   A  [T   I   S   H   D   G   S   D   T   Y   F   R   D]   62
                                CDR 2
301  AAGGGGCTGGAGTGGGTCGCAACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGAC  360

63   S   V   K   G]  R   F   T   I   S   R   D   N   S   K   N   T   L   F   L   Q    82
361  TCCGTGAAGGGCCGATTCACTATCTCCAGAGATAATAGCAAAAACACCCTATTCCTGCAA  420
```
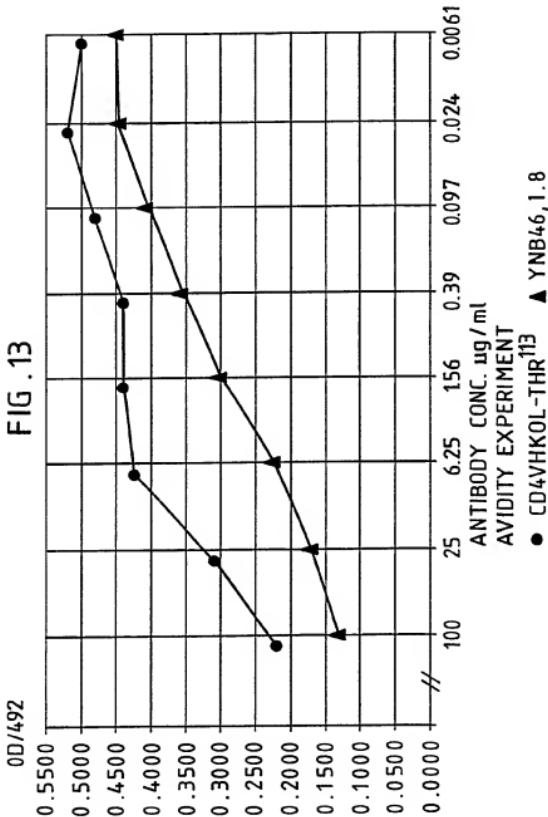
FIG.12 (contd.)

```
                                                                CDR  3
     M   D   S   L   R   P   E   D   T   G   V   Y   F   C   A   R  |Q   G   T   I|        102
83   ATGGACAGTCTGAGGCCCGAGGACACGGGCGTGTATTTCTGTGCAAGACAAGGGACTATA           480
421

     |A   G   I   R   H|  W   G   Q   G   T   T   V   S   S                             122
103  GCAGGTATACGTCACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGGTGAGTCCTTA          540
481

541  CAACCCTCTCTTCTCATTCAGCTTAAATAGATTTTACTGCATTTGTTGGGGGGAAAATGT          600

601  GTGTATCTGAATTTCAGGTCATGAAGGACTAGGGACACCTTGGGAGTCAGAAAGGGTCAT          660

661  TGGGAGCCCGGCTGATGCAGCAGAGACATCCTCAGCTCCCAGACTTCATGCCCAGAGATT          720

              BamHI
721  TATAGGGATCC                                                          731
```

FIG .13

AVIDITY EXPERIMENT
● CD4VHKOL-THR^{113}　　▲ YNB46,1.8

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 21/08, C 12 N 15/13, A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 P; C 12 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[*]	Citation of Document,[11] with Indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO, A1, 9007861 (PROTEIN DESIGN LABS, INC.) 26 July 1990, see page 5; page 10, line 25 - page 14; page 28 - page 30	1-5
Y		1-9
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X	Proc. Natl. Acad. Sci., vol. 86, December 1989, Cary Queen et al.: "A humanized antibody that binds to the interleukin 2 receptor ", see pages 10029-10033, page 10031 right column-page 10033	1-5
Y		1-9
	--	
Y	Nature, vol. 341, October 1989, E. Sally Ward et al.: "Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli ", see page 544 - page 546	1-9
	--	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16th December 1991	0 8 JAN 1992

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT	(CONTINUED FROM THE SECOND SHEET)	
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A	Proc.Natl.Acad.Sci., vol. 87, June 1990, J Sharon: "Structural correlates of high antibody affinity:Three engineered amino acid substitutions can increase the affinity of an anti-p-azophenylarsonate antibody 200-fold ", see page 4814 – page 4817	1

A	Science, vol. 239, March 1988, M Verhoeyen et al.: "Reshaping Human Antibodies: Grafting an Antilysozyme Activity ", see page 1534 – page 1536	1–9

A	Nature, vol. 321, May 1986, P T Jones et al.: "Replacing the complementarity-determining regions in a humanantibody with those from a mouse ", see page 522 – page 525 page 525, left column	1

A	Nature, vol. 328, August 1987, S. Roberts et al.: "Generation of an antibody with enhanced affinity and specificity for its antigen by protein engineering ", see page 731 – page 734	1

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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P,X	WO, A1, 9107492 (CENTRAL BLOOD LABORATIORIES AUTHORITY) 30 May 1991, see page 3	1
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P,X	WO, A1, 9109967 (CELLTECH LIMITED) 11 July 1991, see the whole document	1-9
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		GB-A-	2216126	04/10/89
		WO-A-	89/07452	24/08/89
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		WO-A-	91/09968	11/07/91
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EP-A1- 0403156	19/12/90	CA-A-	2018248	07/12/90
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		WO-A-	91/09968	11/07/91